

PRESERVATION AND INTERPRETATION OF $\delta^{34}\text{S}$ VALUES IN CHARRED ARCHAEOBOTANICAL REMAINS

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Measurement of sulfur isotope ($\delta^{34}\text{S}$) values in charred plant remains has the potential to inform understanding of the spatial configuration and ecology of crop production. We investigated the effects of charring, manuring, oxidation and anaerobic soil conditions on modern cereal grain/pulse seed $\delta^{34}\text{S}$ values, and assessed the effect of chemical pre-treatment on charred modern and archaeobotanical grain/seed $\delta^{34}\text{S}$ values. We used these results to interpret $\delta^{34}\text{S}$ values in archaeobotanical material from Neolithic Çatalhöyük. Our results suggest that $\delta^{34}\text{S}$ values can be reliably preserved in charred grain/seeds but are subject to influence by anaerobic soil conditions, the effect depending on the timing of flooding in relation to S assimilation.

KEYWORDS: SULFUR ISOTOPES, IRMS, ARCHAEOBOTANY, NEOLITHIC, TURKEY, FARMING

Introduction

Plant sulfur isotope values ($\delta^{34}\text{S}$) vary according to local geologic, geographic and soil conditions (Nielsen 1974 Krouse *et al.* 1996; Cortecci *et al.* 2002; Norman *et al.* 2006; Seal 2006; Hoefs 2008; Nehlich 2015). Direct measurement of $\delta^{34}\text{S}$ values in ancient plants could therefore inform about the landscape zone and agronomic conditions in which crops were grown, especially in conjunction with other isotopic measurements (e.g. Ferrio *et al.* 2005; Aguilera *et al.* 2008; Fiorentino *et al.* 2008, 2012; Heaton *et al.* 2009; Heier *et al.* 2009; Riehl *et al.* 2014; Bogaard *et al.* 2013a; Masi *et al.* 2014; Vaiglova *et al.* 2014a; Wallace *et al.* 2015; Styring *et al.* 2017). This study seeks to investigate the preservation of $\delta^{34}\text{S}$ values in charred plant remains, and to understand the causes of variability in $\delta^{34}\text{S}$ values. The objectives of this study are: 1) to test the effects of charring and manuring on $\delta^{34}\text{S}$ values of modern cereal grains, 2) to examine the effect of chemical pre-treatment on $\delta^{34}\text{S}$ values of charred modern and archaeobotanical plants, 3) to determine whether $\delta^{34}\text{S}$ values of charred ancient plant remains were reliably preserved, including experimental oxidation of modern charred plant samples using H_2O_2 , 4) to determine the effects of anaerobic soil conditions on crop $\delta^{34}\text{S}$ values and 5) to apply these methods to examine archaeological material from Neolithic Çatalhöyük, central Anatolia in order to investigate ancient crop growing conditions and as a complement to previous $^{87}\text{Sr}/^{86}\text{Sr}$ isotope measurements (Bogaard *et al.* 2014).

Sulfur isotopes in plants

A variety of sulfur-containing compounds are present in soil, and are taken up by plants in the form of sulfate (SO_4^{2-}) to perform essential growth and metabolic functions (White and Reddy 2009). Plant and soil $\delta^{34}\text{S}$ values vary widely depending on the rate of atmospheric deposition and weathering of sulfur-containing minerals, influence of seawater sulfate and microbial processes (Krouse *et al.* 1996; Seal 2006; Hoefs 2008; Nehlich 2015). Atmospheric sources typically contribute the largest proportion of plant S (Novák *et al.* 2001) but input from mineralization of soil organic matter must also be considered significant before modern pollution. In Europe, atmospheric deposition peaked around 1970, after which sulfur dioxide emission was reduced by legislation; deposition today is down to preindustrial levels of 100-150 years ago (Eriksen 2009). Mineralisation of organic S results in depletion in ^{34}S by 1-5‰ relative to the organic matter (Schoenau and Bettany 1989). Aerosol and rainwater deposition of the relatively high $\delta^{34}\text{S}$ values of seawater sulfate (+20 ‰) can be detected inland up to 30km (Nielsen 1974; Cortecchi *et al.* 2002; Norman *et al.* 2006; Böttcher *et al.* 2007).

Plant uptake of sulfate results in minimal isotopic fractionation (\sim -1.5‰, Trust and Fry 1992; Tanz and Schmidt 2010) meaning that plant $\delta^{34}\text{S}$ values are closely related to the assimilated source sulfate. However, different plant parts have shown different $\delta^{34}\text{S}$ values: in wheat with +1.7‰ for roots, +1.3‰ for stems, +6‰ for leaves and +4.4‰ for grain, the soil sulphate being +3.7‰ (Tcherkez and Tea 2013). In addition to the factors described above, the bioavailable sulfate pool may be strongly influenced by isotopic fractionation in anaerobic soil conditions. Waterlogged soil diffuses oxygen poorly and as bacteria consume available oxygen within hours, the redox potential (Eh) of the soil decreases and other compounds are used as electron recipients instead (Alewell *et al.* 2008; Balakhnina *et al.* 2009; Cook *et al.* 2009; White and Reddy 2009; Husson 2013). Dissimilatory sulfate reduction (DSR) by sulfur-reducing organisms uses sulfate as an electron recipient for redox reactions, producing ^{34}S -depleted sulfide (lower value $\delta^{34}\text{S}$) and leaving the residual sulfate relatively ^{34}S -enriched (higher value $\delta^{34}\text{S}$) (Cook *et al.* 2009). These DSR processes can produce significant (-46 to -40‰) isotopic fractionation between the different soil S pools available to plants (Chambers and Trudinger 1979; Fry *et al.* 1982; Bottrell and Novák 1997; Mandernack *et al.* 2000).

Under anaerobic conditions, plants may incorporate the residual high-value $\delta^{34}\text{S}$ sulfate (pathway B2, Fig. 1). However, the reduced low-value $\delta^{34}\text{S}$ sulfide still remains in the soil and can also be absorbed by plants as sulfide or it can be re-oxidized and incorporated as sulfate. Sulfur uptake from either of these isotopically different sources will depend on a plants' ability to transport oxygen to the root system or to tolerate sulfide toxicity (Fry 1986; Trust and Fry 1992; Spence *et al.* 2001; Finlay and Kendall 2007). As the re-oxidation of sulfide results in minimal isotopic fractionation (Chambers and Trudinger 1979), the sulfate produced by the re-oxidation of sulfide (pathway B3, Fig. 1) will have much lower $\delta^{34}\text{S}$ values than residual sulfate leftover from DSR (Alewell and Gehre 1999; Mörtz *et al.* 1999; Groscheová *et al.* 2000; Mandernack *et al.* 2000; Papadimitriou *et al.* 2006). This situation becomes further complicated when aerobic conditions return (scenario C, Fig. 1) because the re-oxidized DSR products (with significantly lower $\delta^{34}\text{S}$ values) are made available for normal assimilation (pathway C3).

The seasonal effect of aerobic/anaerobic conditions results in changes of 4-11‰ in soil sulfate $\delta^{34}\text{S}$ values, with primarily high-value $\delta^{34}\text{S}$ SO_4^{2-} during wet periods (i.e. largely B2) and low-value $\delta^{34}\text{S}$ SO_4^{2-} in dry periods, derived from ^{34}S -depleted sulfide formed by DSR during wet months (C3) (Mörth *et al.* 1999; Mandernack *et al.* 2000; Eimers *et al.* 2004; Otero *et al.* 2008; Björkvald *et al.* 2009). Consequently, predicting “flooded” plant $\delta^{34}\text{S}$ values relative to those of plants not subject to aerobic/anaerobic cycles is complex since plant $\delta^{34}\text{S}$ values will depend on the timing of plant growth and sulfur assimilation in relation to this cycling.

Preservation of S and $\delta^{34}\text{S}$ values in archaeological charred grain and seeds

Previous experimental work suggests that the charring process preserves a significant component of the original carbon and nitrogen, with minimal isotopic offset (Fraser *et al.* 2013; Styring *et al.* 2013; Nitsch *et al.* 2015). As is the case for nitrogen, a large amount of grain/seed sulfur exists as amino acids (cysteine and methionine), which when charred undergo Maillard reactions with plant starches to produce complex organic molecules; these molecules protect the nitrogen from microbial attack over time (Knicker *et al.* 1996; Silván *et al.* 2006; Styring *et al.* 2013). Since sulfur from cysteine and methionine, and other S-containing compounds, can also become bound in the high-molecular weight products of Maillard reactions, it is also likely to preserve archaeologically.

The potential for biochemical alteration of original sulfur-containing molecules in ancient grains has not been investigated and is an important issue in our study. However, the preservation of organic material, especially charred organic material (“biochar”) has been widely studied and provides background for our new work (Cohen-Ofri *et al.* 2006, 2007; Zimmerman 2010). These studies have shown that organic material can degrade due to biotic (microbial) or abiotic (e.g. chemical oxidation) pathways. Abiotic oxidation is the dominant mode of oxidation in the early stages of degradation of carbon-based biochars (Cheng *et al.* 2006). The exposed surfaces of biochar gain more O-containing functional groups (e.g. carboxylic acid) and become more hydrophilic over time in the presence of chemical oxidants (Moreno-Castilla *et al.* 2000), ozone (Kawamoto *et al.* 2005), and oxygen in air (Cheng *et al.* 2006; Cohen-Ofri *et al.* 2006, 2007). Experimental degradation of organic material has employed bacterial inoculation (aerobic and anaerobic) and progressive oxidation with H_2O_2 (Charrié-Duhaut *et al.* 2000; Lehmann *et al.* 2005; Zimmerman 2010; Olivier 2011).

Organic S in bitumens, especially low-molecular weight compounds (Charrié-Duhaut *et al.* 2000), is lost over time, due to a combination of water evaporation and also biotic/abiotic oxidation (Fedorak and Grbić-Galić 1991; Ohshiro and Izumi 1999). Oxidative cleavage releases low-molecular weight subunits (including sulfones) which are more soluble in water and may be completely removed while also transforming the non-soluble portion of petroleum into more soluble forms similar to humic acids Charrié-Duhaut *et al.* 2000).

Methods

Test 1: Effect of charring and manuring on $\delta^{34}\text{S}$ values

Samples of ripe bread wheat grains (*T. aestivum*, L.), manured and unmanured, were taken from three experimental stations: Askov, Denmark; Rothamsted, UK; and Bad

Lauchstädt, Germany (Körschens and Pfefferkorn 1998; Christensen *et al.* 2006; Rothamsted Research 2006). At Askov and Rothamsted (Broadbalk Winter Wheat) samples derived from experimental plots that were either fertilized with farmyard manure (“FYM”), or unmanured (“NIL”), while at Bad Lauchstädt (Static Fertilization Experiment) manured samples came from plots receiving a lower or higher rate of manuring. Details of the growing conditions are reported in Fraser *et al.* (2011). Random samples of grains were taken from each plot and divided into three aliquots of ~ 50 grains. One aliquot from each plot was left uncharred, while the other two were charred at 230°C for six and 24 hours respectively, in a pre-heated Gallenkamp (London, UK) Plus II electric oven, following the procedure described by Nitsch *et al.* (2015).

Test 2: Effect of pre-treatment on $\delta^{34}\text{S}$ values in charred modern and archaeobotanical material

Laboratory pre-treatment of archaeobotanical remains for radiocarbon dating routinely employs an Acid-Base-Acid (ABA) chemical pre-treatment to remove exogenous carbon. While Vaiglova *et al.* (2014b) recently investigated the effectiveness of pre-treatment methods for plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis, the effect of ABA treatment on $\delta^{34}\text{S}$ is unknown and must be investigated since, in future, samples available for destructive analyses such as $\delta^{34}\text{S}$ measurement may have already been subject to ABA treatment. Charred grain (bread wheat, barley and cereal indeterminate) and pulses (bitter vetch) from medieval (UK) and Roman (UK) contexts was measured to assess the effect of ABA pre-treatment. While the degree of contamination in this archaeobotanical material is unknown, these results were compared to two (uncontaminated) modern charred einkorn samples in order to assess chemical alteration due to pre-treatment. Prior to pre-treatment, each collection of charred grains/seeds was divided into two groups, one of which was subjected to a gentle Acid-Base-Acid pretreatment (identical to “ABA-neutrality” method reported by Vaiglova *et al.* 2014b). Samples were treated with 1M HCl (aq.) at 80°C for 30 minutes (until effervescence ceased), then 0.1M NaOH (aq.) for 25 minutes, before repeating the first acid stage. Samples were rinsed 3x with Milli-Q water (Merck Millipore, division of Merck KGaA, Darmstadt, Germany) between stages. The remaining group was left untreated for comparison.

Test 3: Experimental oxidation of modern einkorn and lentils

Three different experimental oxidation experiments were undertaken (Table 1) under a range of different solution strengths and heating conditions, designed to simulate a progression of oxidation from none (water control) to complete (30% (w/w) H_2O_2 at 80°C for 1 week). A group of ~1000 grains/seeds of organic lentils (*Lens culinaris*, L.), einkorn (*Triticum monococcum*, L., both purchased from Aroma Plantes, Sault, France), and faro/emmer (*Triticum dicoccum*, Bontà della Garfagnana, Italy) were charred for 24h at 230°C. The charred grains were subdivided into aliquots weighing ~ 1.0g (~30 seeds/grains) and transferred to glass tubes with loose screw cap lids for treatment with 50mL H_2O_2 (for details see Table 1). Heat-treated samples were heated to 80°C using a hot block.

Test 4: Variability in $\delta^{34}\text{S}$ values due to anaerobic soil conditions

Test 4A) Irrigated and flooded rice, Spain:

Rice grown in aerobic soil conditions (rain-fed supplemented with irrigation) had significantly lower concentrations of arsenic (As) compared to adjacent plots grown

under conventional flooded conditions, thought to be due to the low Eh potential of the flooded soils allowing greater mobilization of As (Moreno-Jiménez *et al.* 2014). This study analysed $\delta^{34}\text{S}$ values of the same rice aliquots measured by Moreno-Jiménez *et al.* (2014) to determine the relationship between soil Eh potential and rice $\delta^{34}\text{S}$ values, given rice's rhizosphere adaptations to anaerobic conditions. This consisted of three replicate plots of rice grown under a) conventional flooded cultivation, b) the first year of a switch to rain-fed plus sprinkler irrigation, c) the seventh year of the continued rain-fed plus sprinkler regime.

Test 4B) Wild plants, Israel:

Hartman and Danin (2010) recently demonstrated a positive relationship between increasing aridity and $\delta^{15}\text{N}$ values in wild plants collected across a large rainfall gradient in Israel, and noted that plants growing in the streambeds of seasonally flooded wadis had higher $\delta^{15}\text{N}$ than plants growing on adjacent exposed ridges. Here, we measured $\delta^{34}\text{S}$ values of the same aliquots from plants growing in wadis and exposed ridges at four of the locations studied by Hartman and Danin (2010). All plants were collected during the 2007 wet season and included a variety of different plant types from each location (shrubs, dwarf shrubs, C_4 dwarf shrubs, forbs, geophytes). Different locations do not correspond to differences between plant types.

Test 4C) Cereals and wild grasses Konya plain, Turkey:

Modern cultivated cereal and wild grass species were collected from the Konya plain in central Turkey in summer, 2011 (Fig. 2). The typical waterlogging conditions at each sampling location were assessed by observation of the terrain and interviews with local inhabitants. Plants that were waterlogged at the time of collection were distinguished from those in dry locations that were not prone to flooding. The soil substrate was identified using local maps (de Meester 1970; Bogaard *et al.* 2014). Several whole ripe plants were taken from each location, and uncharred cereal/grass grains from multiple plants were analysed.

Test 5: Measurement of $\delta^{34}\text{S}$ values in archaeobotanical samples

Twenty charred archaeobotanical samples of a variety of taxa from Neolithic Çatalhöyük, in the southern Konya plain, central Anatolia (c. 7100-6000 cal BC) (Bayliss *et al.* 2015), were measured for $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values. Different plant parts were analysed depending on the species: cereal grains, lentil seeds, reed culm/stem, nut shell and nut meat. All samples had been previously treated using an ABA protocol identical to Test 2.

Analytical methods

Fresh modern seeds/grains were threshed/dehulled and homogenized using a SPEX (Stanmore, UK) 6850 FreezerMill prior to analysis. Samples that were chemically treated were freeze-dried before homogenization. Charred samples (archaeological and modern) were homogenized in an agate mortar and pestle prior to analysis.

Aliquots from each sample were weighed into tin capsules for analysis with ~2mg V_2O_5 to aid combustion for measurement of $\delta^{34}\text{S}$ values. Analysis of sulfur and nitrogen isotopes was by Continuous Flow Isotope Ratio Mass Spectrometry (CFIRMS). The instrumentation is comprised of an Elemental analyzer (Flash/EA, ThermoFinnigan, Bremen, Germany) coupled to a ThermoFinnigan Delta^{plus} XL isotope ratio mass spectrometer via a ConFlo III interface (ThermoFinnigan, Bremen,

Germany). Sulfur ($\delta^{34}\text{S}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios are reported in per mil (‰) relative to the VCDT and AIR standard respectively. $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values were calibrated using an in-house reference material BROCC-2 (powdered broccoli) with expected delta values of 11.67‰ (calibrated against S-1 and S-2, IAEA) for $\delta^{34}\text{S}$ and 1.5‰ (calibrated against USGS-40 and USGS-41) for $\delta^{15}\text{N}$. For $\delta^{34}\text{S}$ values, the average 1σ reproducibility for mass spectrometry controls for these analyses was $\pm 0.20\text{‰}$. Where samples were analysed in duplicate or triplicate, the average standard deviation was 0.24‰. For $\delta^{15}\text{N}$, the 1σ reproducibility for BROCC-2 was $\pm 0.16\text{‰}$. Statistical calculations were performed using the programming language R (3.1.3).

Results

Test 1: Effect of charring and manuring on $\delta^{34}\text{S}$ values

There are differences in $\delta^{34}\text{S}$ values between sites, as well as differences within sites related to both charring and manuring (Fig. 3, Table S1). In order to assess the effect of charring and manuring on $\delta^{34}\text{S}$ values while accounting for site-level variation, a mixed-effects linear model was used, with site as a random effect, and charring and manuring as fixed effects. All levels of manuring were grouped together as “manured”. The effect of interaction between charring and manuring was also tested, but this effect was not significant ($t[47] = -0.78, p = 0.4381$), and therefore the model without the interaction effect was used because it resulted in a more parsimonious fit (AIC = 95.33 for interaction model, 93.35 for simple model). Although the manured wheat grains had $\delta^{34}\text{S}$ values significantly lower than unmanured grains (0.4‰, $p = 0.0121$), the difference was very small, and similar to the reported analytical uncertainty. There was also a small but significant effect for charring, with charred samples having lower $\delta^{34}\text{S}$ values than uncharred samples (estimated difference = 0.7‰, $p < 0.0001$).

As expected due to loss of free and chemically-bound water (Styring *et al.* 2013), the charred samples have higher %S compared to the uncharred samples ($0.13 \pm 0.02\%$ vs. $0.09 \pm 0.01\%$, Table S1). There was no difference in %S content between manured and unmanured samples.

Test 2: Effect of pre-treatment on $\delta^{34}\text{S}$ values in charred modern and archaeobotanical material

ABA-treatment did not have a measurable effect on the %S content of samples given typical analytical precision (Fig. 4a, Table S2). In both modern charred and archaeobotanical material, $\delta^{34}\text{S}$ values were consistently lower for ABA-treated samples compared to untreated replicates, between -0.4 to -3.1‰, averaging -1.9‰ (Fig. 4b) (multiple linear regression testing for effect of the individual sample and treatment: $\beta = -1.917, t = -4.304, p = 0.005, R^2 = 0.87$). %S was considerably lower in ancient charred samples (of various age and geographic origin) compared to modern charred grain (Fig. 4a; Table S2; see also Test 5). There were no differences in %S between charred archaeological cereal grains (barley, bread wheat) and pulses (lentils, vetches) from the same site.

Test 3: Experimental oxidation of modern charred einkorn and lentils

Experimental oxidation of progressively increasing strength (from 0.1% H_2O_2 20°C, for 1 week, then 1% H_2O_2 at 80°C for 1-8 weeks and finally 30% H_2O_2 at 80°C for 1 week) results in a gradually decreasing %S (see Fig. 5a-c; Table S3). The samples that received the harshest treatment had significant loss of S, and the morphology of

the grains/seeds was severely altered, leaving only an orange residue. Those that received gentle treatment at room temperature did not exhibit any significant loss of S (see Fig. 5b). Samples treated with relatively dilute (1%) H₂O₂ for between 1-8 weeks showed up to 50-60% loss of %S, which is similar to the %S observed in archaeological samples, while the original morphology of the grain for these samples was also preserved. There is no significant relationship between $\delta^{34}\text{S}$ values and %S (multiple linear regression testing for effect of species and %S, for %S: $\beta = -3.2072$, $t = -0.397$, $p = 0.69$, $R^2 = 0.91$, Fig. 8), even when species are considered separately (see Fig. 5a and 5c).

The average difference between the four long-term experiments that included treatment with relatively dilute (1%) H₂O₂ (1-8 weeks), which most closely resembled the archaeological samples, and the water-treated control for both lentil and einkorn, was 0.6‰ (multiple linear regression testing for effect of species and treatment type, for treatment type: $\beta = 0.55$, $t = -1.449$, $p = 0.185$, $R^2 = 0.8422$), a difference which is not significant (95% CI[-0.2, 1.3‰]) based on the small number of replicates.

Test 4: Variability in $\delta^{34}\text{S}$ values due to anaerobic soil conditions

4A) Irrigated and flooded rice, Spain:

Flooded (conventionally grown) rice from Spain had similar $\delta^{34}\text{S}$ values to rice that had been grown in fields sprinkler-irrigated for 7 years ($9.1 \pm 0.6\text{‰}$ for flooded, $9.8 \pm 0.8\text{‰}$ for 7-year Table S4, Fig. 6). Rice harvested after the first year of the transition to sprinkler-irrigation (from flooding) had lower $\delta^{34}\text{S}$ values ($7.7 \pm 1.5\text{‰}$), but differences between treatment groups were not significant ($F[2,6] = 3.18$, $p = 0.11$). The aerobic/anaerobic status was confirmed by the Eh potential of the soil, which changed dramatically between flooded conditions (19 ± 2 mV) and the first year of irrigation (457 ± 14 mV), but remained consistent (429 ± 2 mV) once the irrigation conditions were established (Moreno-Jiménez *et al.* 2014).

4B) Dry wadi and exposed ridge plants, Israel:

Plants growing at the bottom of dry wadis had higher $\delta^{34}\text{S}$ values than those grown on exposed ridges (Fig. 7a, Table S5), by an average of 2.1‰ ($t = -3.14$, $p = 0.004$) although there was a notably wide range of $\delta^{34}\text{S}$ values in exposed ridge samples from location 2 (Ezuz). Plants from wadis had significantly higher $\delta^{15}\text{N}$ values than plants from exposed ridges (Hartman and Danin 2010) but in the subset of samples analysed here, this difference was not quite significant for $\delta^{15}\text{N}$ values, and accordingly the overall correlation between $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values, which is positive at 3 out of the 4 sampling locations (Fig. 7b), was also not significant ($R^2 = 0.018$, $p = 0.35$, for 3 out of 4 sampling locations).

4C) Konya plain, Turkey:

Modern plant samples from the Konya plain were separated by landscape zone, distinguishing between soil substrate and local waterlogging conditions (Fig. 8, Table S6). The results from wild grasses and domesticated cereal species were considered together, since each taxon was sampled from a variety of landscape zones. Samples taken from flooded or habitually flooded contexts (in alluvial and marl/sandplain soil substrates) had significantly lower $\delta^{34}\text{S}$ values ($< 3.5\text{‰}$) compared to samples from non-flooded conditions, with samples from dry alluvial contexts having notably higher $\delta^{34}\text{S}$ values (6.5 to 8.0‰). Unlike Israel, plants associated with flooded conditions had significantly *lower* $\delta^{34}\text{S}$ values compared to non-flooded comparators.

Nitrogen isotope measurements of plants from flooded and non-flooded areas are highly variable, between 3.1 and 9.2‰ in flooded areas and between -2.7 and 9‰ for $\delta^{15}\text{N}$ values in non-flooded areas. There was no correlation between nitrogen and sulfur isotope values.

Test 5: Measurement of $\delta^{34}\text{S}$ values in archaeobotanical samples

The results presented above suggest that charring decreases $\delta^{34}\text{S}$ values by $\sim 0.7\text{‰}$ (standard error 0.1467), while ABA-pretreatment decreases $\delta^{34}\text{S}$ values by 0.4 to 3.1‰ (on average 1.9‰, standard error 0.4456), and the average offset due to oxidation was 0.6‰ (standard error 0.34). Future experimental work would be able to refine the estimates of offsets due to charring or oxidation, while effects due to ABA-pretreatment could be avoided. The Çatalhöyük samples for test 5 were treated using ABA for previous research, and so $\delta^{34}\text{S}$ values can only be compared directly by adjusting for these effects. Considering all of the potential offsets, measured $\delta^{34}\text{S}$ values may need to be adjusted by $+3.2 \pm 0.6\text{‰}$ so that they could be compared with the results obtained from modern samples in the Konya plain presented above (Fig. 9).

The majority of the archaeological material (Table S7) had very consistent $\delta^{34}\text{S}$ values between 3 and 5‰, which – when adjusted to between 6.2 and 8.2‰ – are similar to the modern plant samples grown in dry alluvial conditions (Test 4C) in marls/sandplain and limestone landscape zones. While the majority of the adjusted archaeological $\delta^{34}\text{S}$ values were similar to alluvial contexts unaffected by flooding, some (2/17) of the measured values were significantly lower than 3‰ (unadjusted) and, even when accounting for possible effects of charring and pre-treatment, were similar to values measured in modern plants sampled from contexts that were prone to persistent waterlogging. The two samples with very low $\delta^{34}\text{S}$ values were both wheats (wheat indeterminate and einkorn wheat).

Discussion and conclusions

The key implications of our results are as follows (see Supplementary Information online for more extended discussion of these points).

- 1) Charring has a small but predictable effect on plant $\delta^{34}\text{S}$ values (-0.7‰).
- 2) $\delta^{34}\text{S}$ values in ABA-treated samples are consistently lower than untreated samples. Gentle ABA treatment is likely to alter $\delta^{34}\text{S}$ values to a greater extent than any potential unremoved contaminant would, similar to effects noted for pre-treatment and $\delta^{15}\text{N}$ values (Zimmerman 2010). We recommend limited use of chemical pretreatment for $\delta^{34}\text{S}$ analysis of charred archaeological plant material.
- 3) Unlike N, S in charred archaeological samples is $\frac{1}{2}$ to $\frac{1}{3}$ as abundant when compared to modern charred samples. While this may reflect inherent differences between ancient and modern cultivars, it may also reflect loss of S through microbially or chemically mediated oxidation. Progressive oxidation of modern charred samples with different strengths of H_2O_2 at different temperatures results in %S loss that is comparable with archaeological samples. Chemical oxidation decreased $\delta^{34}\text{S}$ values, but this effect is minimal (0.6‰) for samples that underwent moderate, prolonged oxidation, which were the closest analogy for archaeological

material. This difference was not statistically significant for the small number of samples reported here, but this effect deserves further consideration for the precise application of $\delta^{34}\text{S}$ measurements.

4) In soil, several processes occur concurrently and the isotopic fractionation associated with a particular transformation can be confounded with other effects. Thus, the relationship between anaerobic soil conditions and plant $\delta^{34}\text{S}$ values is complex and it is difficult to disentangle differences in the source soil S due to atmospheric input and the potential effects of isotopic fractionation by soil bacteria. Where non-waterlogging-adapted plants assimilate S during or immediately following flooded conditions $\delta^{34}\text{S}$ values are likely to be higher than comparators not affected by flooding (pathway B2, Fig. 1). In test 4B the extreme aridity likely restricted plant growth (and S assimilation) to the wet season where local flooding conditions persisted in wadi basins. On the other hand, where plants assimilate S only after the return of aerobic conditions, plant $\delta^{34}\text{S}$ values are likely to be lower than unaffected comparators, since plants grown in soil affected by waterlogging may assimilate the low ^{34}S products of DSR (pathway C3, Fig. 1). The plants from waterlogged areas in the Konya plain (test 4C) experienced prolonged flooding and likely restricted growth during periods of excess water, but could have assimilated the low ^{34}S products of DSR when aerobic conditions periodically returned. The potentially wide variability of plant $\delta^{34}\text{S}$ values due to localized flooding conditions may confound attempts to trace geographic origin with plant $\delta^{34}\text{S}$ values directly, but presents an opportunity to understand landscape patterns in relation to flooding in more detail.

5) Measurement of $\delta^{34}\text{S}$ values in ancient crop remains could be useful to identify cultivation in different landscape zones. At Neolithic Çatalhöyük, Turkey, the majority of plant remains sampled had a relatively narrow range of $\delta^{34}\text{S}$ values, and although the comparison with modern landscape zones depends strongly on applying precise offsets due to charring, pre-treatment and oxidation, this group of $\delta^{34}\text{S}$ results are broadly consistent with modern samples from dry alluvial, marl/sandplain or limestone soils. Two wheat samples had lower $\delta^{34}\text{S}$ values, consistent with modern samples grown in (seasonally) waterlogged soils. Overall $\delta^{34}\text{S}$ analysis corroborates other sources of evidence (Bogaard *et al.* 2013b, 2014; Shillito *et al.* 2013; Ryan 2014) for cultivation at Çatalhöyük occurring in the low-lying alluvial/marl zone adjacent to the site. Recent geoarchaeological work (Ayala *et al.* 2017) suggests that this was a dryland anastomising river setting with localised waterlogging; it is therefore plausible that crops were largely (if not completely) protected from such effects.

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Figure captions

Figure 1. Potential differences in plant $\delta^{34}\text{S}$ values due to fractionations associated with sulfate (SO_4^{2-}) transformation during anaerobic and aerobic conditions. (A) “normal” conditions, where soil sulfate is directly assimilated by plants. When anaerobic conditions occur (B) some of the original sulfate pool is available for assimilation (B1) but this quickly becomes reduced to sulfide (e.g. H_2S) through

Disimilatory Sulfate Reduction (DSR). This produces reduced sulfur with extremely low $\delta^{34}\text{S}$ (e.g. -20 ‰) values that coincide with extremely high $\delta^{34}\text{S}$ values (e.g. +20‰) measured in the remaining sulfate. This leftover high $\delta^{34}\text{S}$ value sulfate is available for assimilation by plants (B2), however so is low ^{34}S sulfide, which can be re-oxidized in the rhizosphere (B3), especially of water-adapted plants. When aerobic conditions return (C) the low ^{34}S sulfide can be re-oxidized into sulfate, which is available for assimilation (C3) without significant fractionation.

Figure 2. Map showing location of samples (numbered points) taken for $\delta^{34}\text{S}$ analysis in the Konya plain, south-central Turkey (after de Meester 1970 and Bogaard et al. 2014: Fig. 2). The star indicates the archaeological site of Çatalhöyük.

Figure 3. $\delta^{34}\text{S}$ values of modern bread wheat from three different growing experiments (Askov, Denmark; Bad Lauchstädt, Germany; Rothamsted, UK), comparing manured (black) and unmanured plots. Each point is an individual measurement of a homogenized powder of multiple seeds or grains, with each powder measured in duplicate or triplicate. 1σ reproducibility including all replicate samples was $\pm 0.24\text{‰}$. Rates of manuring are 35-37 t/ha for Askov and Rothamsted, while for Bad Lauchstädt, two levels of manure are compared: FYM1 (20t/ha) and FYM2 (30t/ha). 0h = uncharred/fresh; 6h = heated at 230°C for 6 hours; 24h = heated at 230°C for 24 hours.

Figure 4. a) Comparison of %S by weight for ABA-treated and untreated charred modern cereals and pulses from modern, Medieval (UK), and Roman (UK) contexts. The average and 1σ range of modern charred cereals from test 1 is shown as a shaded region. b) $\delta^{34}\text{S}$ values for different replicates of the same homogenized charred plant sample (cereal and pulse), comparing ABA-treated and untreated samples. 1σ reproducibility including all replicate samples was $\pm 0.24\text{‰}$. Multiple values for Roman cereal (treated and untreated) represent multiple IRMS measurement of the same aliquot.

Figure 5. a) Differences in $\delta^{34}\text{S}$ values for different oxidation treatments with different dilutions of H_2O_2 (w/w or v/v) at either room temperature or 80°C. b) %S with different oxidation treatments. c) Relationship between $\delta^{34}\text{S}$ values and %S for chemically oxidized samples. Regression line and 95% confidence interval (shaded region) also shown. 1σ reproducibility for $\delta^{34}\text{S}$ values including all replicate samples was $\pm 0.24\text{‰}$.

Figure 6 Comparison of $\delta^{34}\text{S}$ values from long-term flooded (conventional) rice, year 1 of the switch to non-flooded (sprinkler-irrigated rice) and year 7 of sprinkler-irrigated rice (Moreno-Jiménez et al. 2014). Each measurement was made of a homogenized powder of several dozen dehusked rice grains, with one measurement from each of the three replicate experimental plots. Samples from the same experimental plot are joined by lines.

Figure 7. a) Differences in $\delta^{34}\text{S}$ values for plants growing in dry streambeds (wadis) or exposed ridges at different sites in Israel. For details of sample collection see Hartman and Danin (2010). b) Relationship between $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values for plants from dry riverbeds (wadis) and exposed ridges in Israel with regression line and 95%

confidence region (shaded area) indicated. $\delta^{15}\text{N}$ values originally published by Hartman and Danin (2010).

Figure 8. $\delta^{34}\text{S}$ values of modern plants sampled from five different landscape zones in the Konya plain, Turkey. Information about the degree of waterlogging comes from observation combined with interviews with local producers.

Figure 9. $\delta^{34}\text{S}$ values measured in archaeological samples from Neolithic Çatalhöyük, compared to $\delta^{15}\text{N}$ values.